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Current status, issues and developments in microalgae derived biodiesel production

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ABSTRACT

Excessive uses of fossil fuels and environmental degradation have forced the scientists to find alternative and clean sources of energy. Biofuels are considered as potential alternatives as they are green in nature and are sustainable energy sources. Biodiesel is one of the most commonly used biofuel due to its fuel characteristics. Several feedstocks can be used to produce biodiesel. However, in recent years, microalgae have emerged as potential biodiesel feedstocks. Microalgae offer advantages over conventional feedstocks. Microalgae have ability to fix atmospheric CO_2 and convert it into sugars, which are then converted into fuel after biochemical processing. Microalgae have high growth rate and accumulate lipids up to 70% in their cell body. They demand less water and nutrients for their growth as compared to terrestrial crops. Despite these advantages, the scale-up applications of microalgae biofuels have some technical limitations. In this study, we have reviewed the overall process of biofuels production from microalgae with a particular emphasis on biodiesel. Critical factors affecting the biodiesel production process including species isolation, species selection, cultivation, harvesting, and oil extraction are discussed. Current research, barriers and developments concerned to each step of biodiesel production process are summarized. New ideas are proposed to improve the growth rate, lipid contents and harvesting efficiency of microalgae. To assess the economic viability of microalgae oil, an economical analysis is presented. Future research trends are also discussed.

Contents

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1. Introduction

In this century, industrialization and imprudent use of natural resources have raised serious concerns over global environment [1–3]. Anthropogenic activities have increased the concentration of carbon dioxide (CO_2), a major green house gas, up to 390 ppm [4–8]. If this trend continues, CO_2 emission is expected to be considerably high in near future. Thus there is an urgent need to neutralize the effect of CO_2 in the atmosphere for sustainable economic growth and to maintain living standards. In this perspective, several techniques can be applied. One obvious technique is to reduce the energy consumption; however, increased population and human life style are the major obstacles to implement this. Storage of released CO_2 can be another solution [9,10]. Technical as well as economical barriers limit this application also. CO_2 mitigation through natural sinks is a long-known process. Photosynthesis is natural process in which plants use CO_2 as a carbon source and convert it into carbohydrates [11–13]. Carbohydrates can be converted into fuel, called biofuels, through chemical or biochemical processing. Currently, biofuels are being promoted to displace fossil fuels [14–16].

Biofuels have some advantages over traditional fuel sources [17]. They are sustainable and environmentally friendly. Biofuels are derived from natural resources, which are versatile in nature. Based on the feedstocks, biofuels are categorized as 1st generation, 2nd generation and 3rd generation biofuels. Biofuels derived from crop plant such as jatropha, almond, barley, camelina, coconut, copra, fish oil, groundnut, laurel, oat, poppy seed, okra seed, rice bran, sesame, sunflower, sorghum, wheat soybean, rapeseed, karanja are termed as 1st generation feedstocks [18,19]. Biofuels production from such feedstocks faces criticism due to food versus fuel dilemma. Animal fats and waste cooking materials are also used to produce biofuels, known as 2nd generation feedstocks. However, these feedstocks do not have stable supply to fulfill future energy needs. Alternatively, microorganisms, called 3rd generation feedstocks, can be used. A wide variety of microorganisms have been indentified which serve as sink for CO_2 and produce biofuels. They are used to produce methane, bio-hydrogen, biodiesel, and bio-ethanol by subjecting them to different biological conditions. Fig. 1 shows the processes of biodiesel production from various feedstocks.

As noted earlier, biodiesel production from 1st and 2nd generation feedstocks have some ethical and sustainability issues [20]. Recently, the most promising choice for biodiesel production is microalgae. Microalgae have advantages over other feedstocks. Microalgae have high photosynthetic efficiency than terrestrial crops. They grow 100 times faster than other plants. They can give biomass yield of 15–25 t/ha/acre, which is much higher than soybean (0.4 t/ha/year), rapeseed (0.68 t/ha/year), oil palm (3.62 t/ha/year) and jatropha (4.14 t/ha/year) [21–24]. Microalgae have ability to use atmospheric CO_2 as a carbon source. They can fix CO_2 at higher rate than other plants. Microalgae fix CO_2 and convert it into value added products such as vitamins, lipid, protein, bio-ethanol, and bio-hydrogen. Besides distinct advantages of microalgae there are some technical limitations in their scale-up applications [25]. For economical biofuels production from microalgae, we need to select such species of microalgae which have high growth rate, probably less than one day. Also, high lipid yield ($>70\%$) is desired. There is a tradeoff between growth rate and lipid yield. Species growing fast accumulate less lipids. On the other hand, contamination is a serious issue for outdoor microalgae cultivation. Harvesting, which pose 30% of total cost of biodiesel production from microalgae itself is a complex process. No universal technology has been introduced so far for economical microalgae harvesting. Lipid extraction is another necessary step of biodiesel production process. Thus it is worthy to investigate each step of biodiesel production process in detail. In this article, we have reviewed the critical parameters which influence the biodiesel production process. A detail discussion about each parameter is provided to identify the problems which incur in biodiesel production process. This information might be helpful to set future research goals for sustainable microalgae biofuels.

2. Rationale of biodiesel production from microalgae

Continuous increase in human population, living standards, and energy consumption has led to excessive use of fossil fuels. [5,26,27]. According to an estimate, the existing fossil fuel reserves will exhaust in next 60–80 years [1,12,28]. The depletion of fossil fuels would put enormous pressure on the global economy [27]. Thus finding renewable and sustainable sources of energy is one of the most challenging issues of this century [14,29,30]. Biofuels production is a promising choice to overcome these challenges [31]. The most common biofuels are: bio-ethanol, biodiesel, and bio-hydrogen. Among these, biodiesel is an attracting option because of its high energy density, low NO_x and SO_x emission after combustion, and its compatibility with existing vehicle engines without modification. Biodiesel is a mono-alkyl ester of fatty acids. It is produced by the reaction of triglycerides with alcohols. Biodiesel can be produced from edible sources, called 1st generation feedstocks [16,18]. High nutrients and water demand,

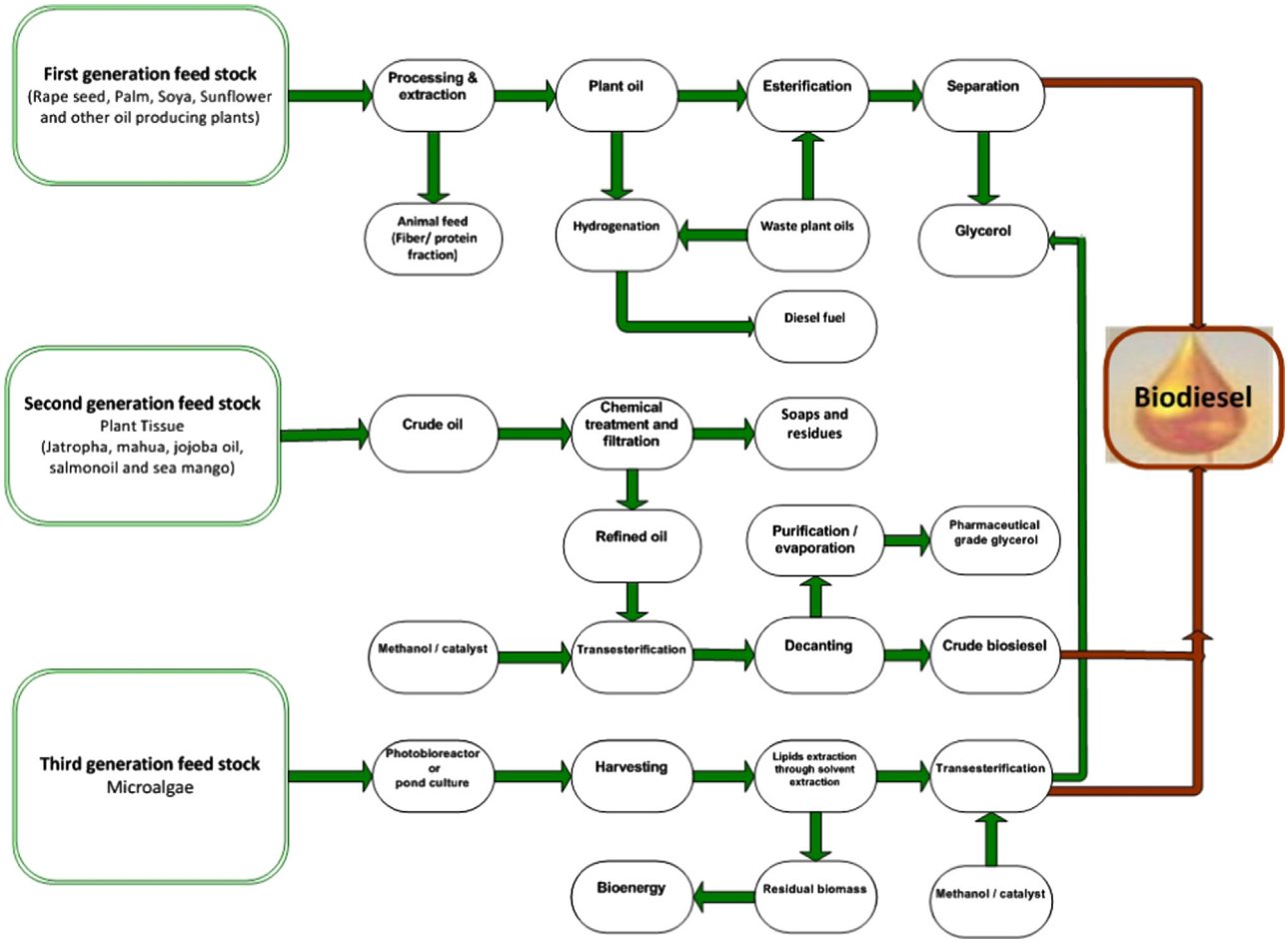

Fig. 1. The processes of biodiesel production from various feedstocks.

and also food insecurity have limited their use for biodiesel production. Non-edible sources like animal fats, waste cooking oil, and cellulosic biomass are other sources of biodiesel [19,32,33]. The sustainability of biodiesel from such sources is a major bottleneck towards their commercialization [17,22,34].

Recently, microalgae are being considered as potential feedstocks for biodiesel production. Microalgae have distinct advantages over other feedstocks. Microalgae consume less water and nutrients to grow as compared to terrestrial crops. Microalgae can give twenty times more productivity (in terms of oil) than oilseed crops. Microalgae grow fast and their doubling time ranges from 3.5 h to 24 h. High growth rate results in high biomass yield in short time. Considering the high growth rate of microalgae, it has high cropping intensity than other crops. They can grow either in domestic or industrial wastewater, using nitrogen and phosphorous as nutrients sources. Microalgae can use municipal or agricultural runoff. Simultaneous growth of microalgae and bacteria can degrade black oil. Microalgae can treat wastewater by ion exchange. They have negative charge on their surface which can adsorb with positively charged compounds present in wastewater. Moreover, it can adsorb industrial flue gases and atmospheric CO_2.Thus it is a sink for greenhouse gases. It converts CO_2 into organic molecules (carbohydrate, protein, and lipids) through photosynthesis.

3. What are microalgae?

Microalgae are unicellular micro-organisms. Microalgae are categorized as prokaryotes and eukaryotes. Organelles are the major difference between prokaryotes and eukaryotes. Prokaryotes do not possess chloroplast, mitochondria and nuclei [28,35] but they contain *chlorophyll a* and high protein contents [36]. Microalgae are further divided into different groups based on their taxonomy, including blue-green, green, yellow-green, red, brown, and golden algae. There are more than 50,000 species of microalgae. Different microalgae species thrive in diverse environment, freshwater, brackish water, and also in wastewater. Microalgae can also be categorized based upon carbon supply [11]. Some microalgae use inorganic carbon such as CO_2, are known as *autotrophs*. Autotrophs perform photosynthesis using light as energy source while heterotrophic microalgae use organic carbon [33,37,38]. There are some species which can use both, organic and inorganic carbon sources, are called *mixotrophs* [39].

4. Microalgae biodiesel production process

The whole process of biodiesel production consists of strain isolation and selection, cultivation, harvesting, drying, lipids extraction and finally the biodiesel production. Fig. 2 shows the entire chain of biodiesel production process.

4.1. Species isolation and selection

Isolation of robust species of microalgae is vital for efficient biodiesel production. As discussed earlier, microalgae thrive in diverse environment like freshwater, brackish water, saline and hyper-saline environment [40]. Currently, research is being relied

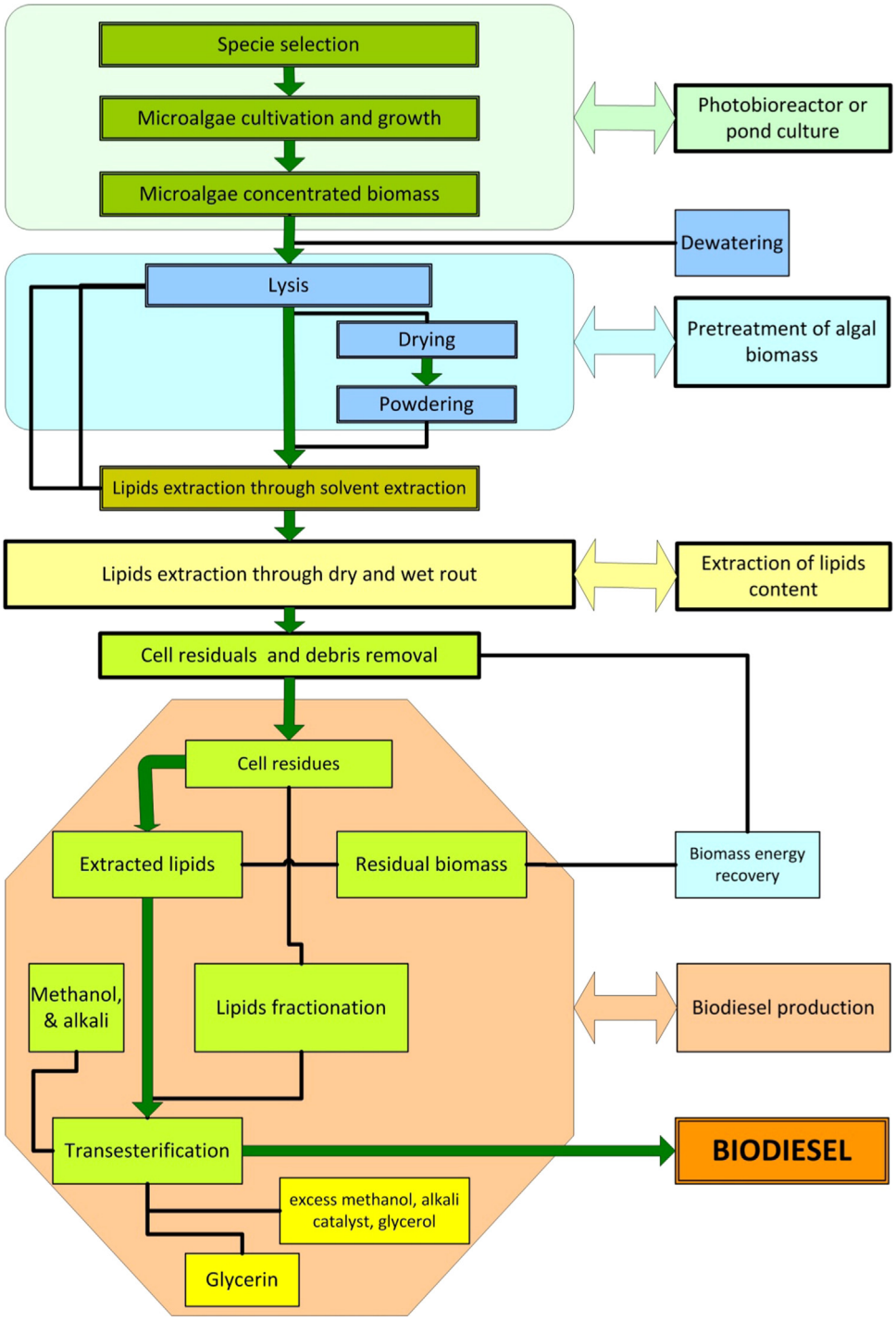

Fig. 2. An illustration of microalgae based biodiesel production process.

on already available species [41]. Georgianna and Mayfield et al. claimed that many of microalgae species have lost their original properties due to their continuous cultivation over years [42]. Basic information about these species is lacking. Therefore, it is important to isolate new species of microalgae. Up to date information about their basic characteristic should also be available. Species isolated from local habitat have high tendency to face environmental stress [43]. Growing microalgae in new environment is challenging. As, they take time to combat with the environmental changes. The cells growing in natural system may lack some metabolites which promote the growth of microalgae. Also, the cells may avail diverse nutrients condition in natural system which is not available at lab scale. Species growing in less saline land are likely to produce more lipids, as lipids are accumulated in nutrients deprived condition. The isolation and selection of microalgae depends on its use. For example, species collected from contaminated sites are favorable for waste water treatment [44]. As these species would be tolerant to the contaminants. Likewise, species isolated from oils station would have ability to accumulate high oil. In fact, these species use oil as a food and store it in their body.

The selection of right microalgae species need to be evaluated on the basis of certain parameters. Generally, the selected microalgae species should be resistant to the environmental changes

like nutritional level, temperature, carbon supply, light, pH, and bacterial contamination. They should have culture stability, growth physiology, and ability to grow at large scale [20,45]. Above all, growth rate and lipids contents are the most crucial factor for species selection. Species with high growth rate can significantly reduce the cultivation time, and thus, cultivation cost. Likewise, high lipid contents can attribute to high biodiesel yield. The composition of lipids is important whilst selecting microalgae. Lipids quality is another factor to select right species of microalgae. Microalgae producing polar and neutral lipids are preferred. *Chlorella vulgaris* and *Chlorella protothecoides* are identified to produce such lipids [4]. Microalgae selection depends on the set objective. For example, *Botryococcus braunii* is rich in oil content (75%) while *Nannochloropsis oculata* shows the highest lipid productivity (142 mg/L/day), and *Scenedesmus sp.* is appropriate specie for carbon-fixation (10%) [46].

4.2. Cultivation

Cultivation of microalgae is a major step in biodiesel production process [47]. Microalgae can be cultivated using various techniques (based on carbon source). Among them, phototrophic cultivation is the most common [48,49]. In phototrophic cultivation, microalgae fix CO_2 in the presence of light and accumulate organic compounds. This simple reaction can be represented by the following equation:

$$2H_2O + CO_2 + \text{light} \rightarrow CH_2O + O_2 + H_2O$$

Photosynthesis is mainly driven by two factor, light and carbon source. Light is useful for carbon fixation, the growth rate and biomass productivity [50]. In natural system, all incoming sun radiations are not available for photosynthesis. Gases in the atmosphere absorb 30% of incoming radiations. *Chlorophyll a* in microalgae can absorb radiation of wavelength 400–700 nm. What is worse, chlorophyll can not absorb more than 30–40% of incoming radiations. More detailed study about light absorption can be found in William et al. article [35]. In-efficient absorption of light can hamper photosynthesis. Microalgae can only grow under high enough light intensity. The level of high intensity is species specific. For example, the light saturation point is 185 $\mu mol/m^2/s$ for *Phaeidactylum cruentum*, 200 $\mu mol/m^2/s$ for *Porphyridium cruentum*, and 150 $\mu mol/m^2/s$ for *Aphanothece microscopia*. Sharma et al. found three times more growth rate at 50 $\mu mol/m^2/s$ as compared to 20 $\mu mol/m^2/s$ [4]. High light intensity can halt photosynthetic process, called *photo-inhibition* [7]. In addition to light intensity, the light color also influence pigment absorption. Microalgae can use only a limited spectrum to perform photosynthesis if white light is used. Red light is proved efficient than the others. Red light is appropriate to excite light pigments, *chlorophyll a* and *b*. Blue light contains 40% more energy than the red. Blue light is not suitable for the photosynthesis. Blue light is used for genes transcription and enzyme activation. Light frequency influence microalgae growth. Wu et al. have suggested growing microalgae on dark/light frequency of 1 Hz. Growth rate changes with photoperiod [51]. A photoperiod of 12:12 (dark: light) gives high growth rate. Jacob-Lopes et al. evaluated the effect of photo-period on growth rate, biomass composition, lipids yield and CO_2 fixation [36]. The biochemical composition of biomass and CO_2 fixation is different for each photoperiod. Keeping the cells in dark condition can halt photosynthesis. In dark condition, respiration starts and 25% of total biomass can loss [52]. Microalgae growth varies with the type of light source. Conventionally, tubes lights and bulbs are used as light sources for microalgae cultivation. Low-cost and efficient light sources can ensure the economical cultivation of microalgae. Yeh et al. tested the effect of light source on microalgae growth. They used tungsten lamp, fluorescent lamp, and fluorescent helix lamp [53]. Fluorescent lamp showed the highest lipid productivity. As compared to these light sources, light emitting diodes (LEDs) are, however, efficient and cheap. According to one study, LED can decrease power consumption up to 50%. LED show high conversion efficiency, generate low amount of heat, and are user friendly. The electricity consumption of LED is lower (20.16 KW/h) than conventional light sources (40.32 KW/h). The added advantage of LED is its narrow spectrum (20–30 nm). The color of LED light also impact microalgae cultivation. Red and blue LED show relatively high efficiency than the others. Light colors carry different levels of energy. Green light (at 550 nm) contains 20% more energy than red, and 15.5% less than blue light [54]. Das et al. tested the effect of different colors (blue, white, green, and red) on *Nannochloropsis sp*. Blue color resulted the highest growth rate while the lowest growth rate was observed using red LED. Basically, blue light has shorter wavelength as compared to red light. So, shorter wavelength has positive impact on the growth rate.

Optical fiber is the alternate light source. In optical fiber, the light is guided by plastic fiber to microalgae culture. Microalgae cells illuminated by optical fiber experience uniform light exposure throughout the culture. Optical fiber does not produce heat and have high surface to volume ratio. The unique advantage of optical fiber as compared to other light source is its use inside the photo-bioreactors. In externally illuminated photo-bioreactors, a large portion of light can not pass through the thick wall of photo-bioreactor [55]. Thus microalgae cells can avail only a limited amount of light. The optical fiber, being immersed into microalgae culture, provides more light to the cells and increases photosynthetic efficiency. The energy consumption of optical fibers is much lower (1 KWH) than LED (20.156 KW/h) and artificial light (40.32 KW/h). Song et al. found that microalgae show high hydrogen yield using optical fiber as internal light source [56]. Light sources can be affordable for microalgae cultivation at lab scale. The mass cultivation of microalgae, however, is not economically supported if artificial light source is used. The ultimate solution is to harvest sunlight, which is abundant and contains full spectrum of light to be used by microalgae [57]. The major obstacle in using sunlight is the variation in day/night duration, season, and insolation intensity. Chen et al. have proposed one solution [58]. They viewed to integrate both, artificial and natural light sources [59]. In day time, electricity can be generated using solar panels which can be used to illuminate photo-bioreactors at night time. Further exploration of this idea is needed to assess its cost-effectiveness.

4.2.1. Carbon source

Carbon is an essential element of microalgae growth. Microalgae have ability to use both, organic and inorganic carbon sources. Autotrophs use inorganic and heterotrophs consume organic carbon as a food source [60]. Autotrophs fix carbon in the presence of light called photo-autotrophs. CO_2 fixation depends on light energy. A light energy of 1338 KJ is required to fix one carbon atom; however, photosynthetic efficiency increases with an increase in CO_2 level. Fogg et al. found an increase in carbon compounds excretion under high light and low carbon concentration [61]. The growth rate and biomass productivity of microalgae increase at high CO_2 level (1–15%). *N. oculata* showed high biomass productivity (0.48 g/L/day) and lipid content (29.7%) at 2% CO_2 level. In dense microalgae culture, the cells suffer light limitation more instead of carbon. In dense microalgae culture, pH, dissolve oxygen, chlorophyll, and biomass yield decrease [37,41,62]. It is viewed that high CO_2 does not necessarily increase the growth rate. Photosynthesis efficiency improves with time due to the adaptation of CO_2 in microalgae culture [63]. Some microalgae species show growth inhibition

at elevated level of CO_2. CO_2 accounts 60% of the total cost of nutrients. The cost of CO_2 can be reduced by using flue gases. Microalgae show growth rate using flue gases. CO_2 can affect lipid content of microalgae. The cells produce poly-unsaturated fatty acids under high CO_2 concentration [64]. A 10–15% increase in CO_2 supply can enhance the lipid content up to 6% [58].

Generally, CO_2 is directly supplied into microalgae culture from the atmosphere. As CO_2 has low solubility in water, therefore, continuous pumping is required to ensure its availability over entire cultivation period. Pumping is carried out through air pump which poses additional cost on cultivation. Another associated problem with CO_2 supply is the decrease in pH. In early growth phase, CO_2 interact with water, forms H_2CO_3 and pH decreases [65]. To overcome this limitation, high concentration of inoculum should be provided. At high inoculum rate, the cells consume all of the CO_2 available, and reaction of CO_2 with water does not take place [66]. The consumption of chemical constituents of CO_2 vary with microalgae species. For example, *Scenedesmus* prefer to use HCO_3 and *Chlorella* only consume dissolved CO_2 [11]. Yeh et al. explored the consumption of CO_2 and HCO_3 by *C. vulgaris*. They used Na_2CO_3 as carbonate and $NaHCO_3$ as bicarbonate. *C. vulgaris* showed high growth rate using $NaHCO_3$ as compared to Na_2CO_3. The interaction of CO_2 and water forms three species (1) carbonic acid (2) bio-carbonate (3) carbonate [67]. Bicarbonate is transmitted to the cell through cell wall [53]. It is consumed by the cells after converting into CO_2. Studies reveal that the cells show high biomass yield and lipid content using bicarbonate as a carbon source. Sodium bicarbonate and methanol can be alternate carbon sources. Microalgae can transform these carbon sources into CO_2. High solubility and easy access of CO_2 to microalgae promote their growth rate. Methanol is an effective carbon source for microalgae. Methanol is derived from plant. Microalgae convert methanol into formaldehyde, formic acid, and finally into CO_2. The cells take up released CO_2 to promote their growth rate. CO_2 evenly distributes in the culture, and easily available to the cells. These distinct features of methanol might help microalgae to grow fast. Studies prove growth stimulation of microalgae under high light intensity and low methanol concentration. Kotzabasis et al. found an increase in growth rate of microalgae by adding 5% methanol [68]. Despite added advantages of methanol, it is not cheap to use for microalgae cultivation. Thus there is need to either find cheaper carbon sources or increase CO_2 solubility by adding a catalyst.

The issue of CO_2 solubility can be resolved to some extent by controlling its bubble size. Large sized bubble (1–2 μm) has high velocity and reach to the top of water surface (in a photo-bioreactor) immediately. Therefore, CO_2 is neither fully mixed nor consumed by microalgae [34]. Small sized bubbles (<1 μm), however, move slowly and burst within the water surface [58]. Resultantly, CO_2 remain in water surface and available to microalgae cells for a long time. A detailed description of this phenomenon can be found in Lam et al. article [67]. The application of micro-bubbles has a limitation. Micro-bubbles produced in large quantity create cavitations in the medium, and make the environment (within the culture) cloudy. Under this phenomenon, the cells can not capture light efficiently. This limitation can overcome by generating bubbles in one unit and pumping them into the photo-bioreactor, rather than carrying it in a single unit. Oxygen supply is a rate limiting step of CO_2 fixation. High oxygen concentration decrease the number of CO_2 acceptors. This also happens at high light and low carbon concentration [63]. Oxygen supply or aeration is critical for economical microalgae cultivation [69]. Conventionally, the research is focused to correlate microalgae growth with CO_2 concentration rather than observing the activity of CO_2. The activity of CO_2 refers to how it is converted into different species (HCO_3, CO_3) and what is its relationship with pH. High concentration of HCO_3 had some toxic effect on the growth due to increase in pH. Also, it should be noted which chemical constituents of CO_2 are dominant in controlling pH.

In addition to CO_2, some microalgae use organic carbon source called heterotrophs [70].They use organic molecules as a substrate and energy source [71]. Generally, heterotrophic organisms consume the food which is prepared by others [72]. Microorganism in heterotrophic condition can also induce photosynthesis by chlororespiration system. A comparison of auto and heterotrophic cultivation of microalgae is presented in Table 1. A biomass yield of 50–100 g/L is possible in heterotrophic condition. Ideal heterotrophic microalgae species must be robust in nature, resistant to the environment, grow fast and produce high biomass. Heterotrophic cultivation is carried out under dark. Therefore, it is easy to maintain heterotrophic cultivation at large scale without depending on light supplement. Heterotrophic microorganism can use organic carbon present in wastewater. This way dual purposes can be served at a time i.e., microalgae cultivation and wastewater treatment. Microalgae can use glucose, acetate, glycerol, fructose, sucrose, lactose, galactose and mannose as organic carbon sources [25,57]. Some studies have also reported to use powder hydrolysate. Microalgae with powder hydrolysate showed biomass yield of 2 g/L/day and lipid content of 3700 mg/L/day. Glucose is widely used carbon source for microalgae. Glucose has more energy content than other carbon sources. Perez et al. have explored the mechanism of glucose consumption in microalgae [73]. Guedes et al. have found changes in cell size, lipids, protein, and chlorophyll by the use of glucose [74]. Oxygen is a key factor to grow heterotrophic microalgae at fast rate. Glucose consumption is somewhat linked with oxygen supply. The consumption of glucose increases the concentration of dissolved oxygen. Dissolve oxygen can be an indirect measure of glucose consumption [55,71]. Munoz et al. view that additional dose of the substrate is required at every 10% increase in dissolve oxygen. The optimal glucose concentration for *Scenedusmus acutus* and *Nitzschia laevis* is 1 g/L and 40 g/L, respectively [39,60]. Xu et al. used corn powder hydrolysate as an organic carbon source instead of glucose [75]. They obtained the cell density of 15.5 g/L and lipid contents of 55.2%. Gao et al. used sweet sorghum juice as an alternative to glucose [29]. The maximum cell density and lipids were 5.1 g/L and 52.5% respectively. The lipid yield was 35.7% more than using glucose (1.2 g/L/day). Further increase in biomass yield was observed by adding yeast extract. They compared the viscosity, flash point, heating value of biodiesel produced by *C. protothecoides* with diesel fuel. All of the properties were in line with ASTM standards. Lin et al. grew *Chlorella zonfingiensis* in heterotrophic and autotrophic conditions [64]. The lipids produced in heterotrophic condition were higher than the autotrophic condition. Moreover, the cells in heterotrophic culture produced neutral lipids while autotrophic condition resulted membrane lipids and phospholipids. It should be noted that neutral lipids are better in quality than other lipids for biodiesel production. Myerse et al. have demonstrated that the cells prefer to use CO_2 in the presence of light and organic carbon [76]. Here we have presented basic information regarding the

Table 1
A comparison of phototrophic and heterotrophic culture system.

Phototrophic	Heterotrophic
Inorganic carbon source	Organic carbon source
Cheap	Expensive
Low chances of contamination	Prone to contamination
High growth rate	Slow growth rate
Can not grow without light	Can grow without light
High capital investment	Low capital investment
Low lipid yield	High lipid yield

uptake of glucose in microalgae culture. More detail about glucose and other organic carbon sources (acetate, glycerol) can be found in Perez-Garcia et al. article [73].

Heterotrophic cultivation is expensive if commercially available organic substrates are used. The use of natural organic compounds can significantly reduce its cost. Literature shows that microalgae species such as *C. vulgaris*, *Scenedesmus acutus*, *C. protothecoides*, *Chlorella saccharophila*, *Chlorella sorokiniana* can grow in heterotrophic culture. Contamination is another issue in heterotrophic cultivation [44]. It can be controlled by sterilization and adopting aseptic techniques. Sterilization is not economically supported for mass cultivation of microalgae. Contamination can be controlled by optimizing substrate concentration. The optimal substrate concentration (ranges from 1 g/L to 25 g/L) is species dependent. High initial substrate sconcentration causes other bacteria to grow. Under optimized substrate concentration there are less chances for the undesired bacteria to grow due to limited nutrients availability. Huang et al. have proposed to increase cell density in heterotrophic cultivation by adopting fed-batch culture, chemostat culture, and membrane cell recycle system [77]. So far, limited studies have been carried to promote heterotrophic cultivation at industrial scale. For commercial application of biodiesel, process mechanism, biochemistry, and phycology of heterotrophic microalgae must be explored further.

4.2.2. Other nutrients

Microalgae consume a wide variety of nutrients to grow. Carbon, nitrogen and phosphorous are essential elements, whereas manganese, magnesium, cobalt, calcium, sulfur, iron, potassium, sodium, and hydrogen are used as trace nutrients [78,79]. The fate of carbon has been already discussed in previous section. The effect of nitrogen and phosphorous is presented in the following section.

Nitrogen regulates protein synthesis and growth metabolites of microalgae. They can use nitrogen in the form of nitrate, nitrite, ammonia, and urea. The form of nitrogen uptake is species dependent; however, majority of the microalgae species prefer to use ammonia as a nitrogen source. The nitrogen uptake alters the pH of microalgae culture which influences the growth rate. Experiments show that nitrate uptake increases the pH. In theory, the assimilation of one mole of nitrate produces one mole of OH^- [56], following the reaction below:

$$1.0(NO_3)+5.7(CO_2)+5.4(H_2O)\rightarrow(C_{5.7}H_{9.8}O_{2.3}N_{1.0})+8.25(O_2)+1.0(OH^-)$$

The evolution of oxygen during nitrate or nitrate uptake depends on the type of microalgae species. Oxygen evolution can not be considered as an ultimate indicator of nitrate assimilation. Microalgae assimilation is catalyzed by the presence of CO_2 and light. Increase in nitrate assimilation can also be observed in the presence CO_2 only. Nitrate uptake has linear relationship with medium pH and microalgae growth. High uptake of nitrate can raise the medium pH above 10 which is not favorable for microalgae growth. Thoreson et al. emphasized that nitrate uptake is influenced by medium pH. Basically, H^+ and OH^- flux affect the metabolic process [80]. Nitrate depletion increases the viscosity of microalgae medium and produce polysaccharides [81]. Thomas et al. observed an increase in carbohydrate contents in nitrogen starved condition [41]. In early stationary phase, the cells growth was influenced by nitrate. Nitrate uptake is catalyzed by providing light. Light-induced chloroplast converts nitrate into nitrite by reducing ferredoxin, pyridine, and flavo-proteins [76].

Microalgae prefer to use ammonia than nitrate as a nitrogen source [82]. The uptake of ammonia produces H^+. One mole of ammonia produces one mole of H^+ following the reaction below:

$$1(NH_{4^+})+7.6(CO_2)+17.7(H_2O)\rightarrow(C_{7.6}H_{8.1}O_{2.5}\,N_{1.0})+7.6(O_2)+15.2(H_2O)+1.0(H^+)$$

High uptake of ammonia lowers the medium pH (<6.0) at which microalgae can not grow. Therefore, pH adjustment is essential to use nitrate or ammonia as a nitrogen source. Microalgae species with high growth rate use ammonium as primary nitrogen source than nitrate [24,83]. Tam et al. found that microalgae show high growth rate at 20 mg/L of ammonia [82]. Higher concentration retarded the growth. Microalgae can consume urea as a nitrogen source. Sakamoto et al. have claimed urea as a better nitrogen source than any other. Urea assimilation requires less energy than nitrate because it exists in reduced form [84]. Microalgae produce CO_2 during ammonia uptake. Released CO_2 is helpful for microalgae growth. [9]. As compared to other nitrogen sources, urea can pass through plasma membrane easily. The cells use urea by converting it into ammonia and bicarbonate. Majority of the microalgae species consume nitrogen in the following order: nitrate > nitrite > urea.

Phosphorous is an important constituent of microalgae cultivation. It is an essential element in energy conversion and transferring information in microorganisms. In the absence of phosphorous, microalgae produce more oxygen [85]. Microalgae show high nitrate uptake in phosphorous depleted medium [85]. Cell activities like metabolism and photosynthesis are greatly affected by phosphorous assimilation. The optimized concentration of phosphorous can increase the growth rate of microalgae. The uptake of nitrogen and phosphorus is complex in microalgae cultivation. They show interactive effect on microalgae growth and lipid synthesis. The optimization of either of them can be misleading. Therefore, their simultaneous effect must be investigated. In this regard, nitrogen to phosphorous ratio (N/P) is an appropriate parameter to consider. Smith et al. have demonstrated that the cells suffer nitrogen limitation at $N/P>1$ and phosphorous limitation at $N/P<1$ [86]. An N/P value of 20:1 is the best suited for microalgae. Microalgae demand high N/P value in exponential phase and low in stationary phase. High N/P implies that microalgae efficiently consume nitrogen and phosphorus to develop cell biomass. Low N/P in stationary phase assists to accumulate more lipids [52]. Applying different levels of N/P values in stationary and exponential phase can give three times more lipids than applying their same level in both phases. The investigation of nitrogen and phosphorous supply in the presence of CO_2 is an important aspect of microalgae cultivation. CO_2 supply greatly influences the uptake of nitrogen and phosphorous. Therefore, carbon to nitrogen ratio, and nitrogen to phosphorous ratio needs to be optimized simultaneously. The optimized use of trace elements can improve microalgae cultivation [87]. Nasr et al. found 37.9% increase in cell biomass by using molybdenum [88]. Cobalt increased the cell biomass by 30.8%, manganese 20.7%, and boron 27.6%. Liu et al. checked the effect of iron on biomass and lipids production [78]. They found 3–7 fold increase in biomass production at 0.12 mol/L of $FeCl_3$ as compared to control; however, it had no significant effect on lipid production.

4.3. Other factors affecting microalgae cultivation

Growth medium, temperature, dissolve oxygen, turbulence/mixing, pH, medium composition, and salinity also affect microalgae cultivation.

Microalgae can grow in a wide range of temperature (5–35 °C) [84]. In natural system, the solar radiations which serve as light source increase the medium temperature. A variation in medium temperature depends on pond depth. Microalgae growing in shallow ponds would face high but homogeneous temperature throughout the culture [50]. In deep ponds, microalgae experience different temperature long the pond depth. Continuous mixing can overcome this limitation. Converti et al. found two-fold increase in lipid content of *N. oculata* by applying optimized temperature conditions [45]. Sakamoto et al. measured the doubling time of *Synechococcus sp. PCC 7002* at different temperatures [84]. The

shortest doubling time (3.5 h) was found at 38 °C. To control medium temperature, Raul et al. have suggested to grow microalgae species together, having similar growth characteristics but different optimum temperatures [44]. *Rhomonas sp*. showed high growth and produce the maximum carbohydrates, lipids, chlorophyll and protein at 25–27 °C. *Chaetoceros sp.* showed enhanced growth at 33 °C and 35 °C [89]. For high lipids yield, the optimum temperatures for *N. oculata* and *C. vulgaris* are 25 °C and 30 °C respectively [45]. *Euglena* cells showed the highest doubling time at 27–31 °C [90]. The optimum temperature for *Monoraphidium sp. SB2* growth is 25–35° C [91]

Oxygen is also a limiting factor of microalgae cultivation. Oxygen is consumed during microalgae respiration and released when photosynthesis takes place. Oxygen produced during photosynthesis can oxidize several enzymes to form oxygenic byproducts which suppress microalgae growth.

The optimal mixing or turbulence is required for homogeneous distribution of gases (oxygen and CO_2), light, and temperature. Slow mixing can generate dark stagnant zone in microalgae suspension. Slow mixing can generate anaerobic zones, which pose deleterious effect on the growth. On the other hand, high mixing can damage viability of the cells due to shear stress. Mixing can be carried out by gas injection, pumping or by mechanical stirring. Among these, mechanical stirring is effective but it cause higher hydrodynamic stress than the others. To overcome this limitation, baffles are designed which control the mixing pattern. Mechanical mixing can be furnished by propeller, air diffuser, perforated tubes, and air jets [83]. Careful selection of a mixing device can increase the biomass productivity up to 75%. Gudin et al. proposed to use airlift system for efficient mixing [92].

pH alters the metabolic activities of microalgae. pH has direct relationship with nutrients uptake. The uptake of CO_2 and nitrate increase pH, while ammonia shows the opposite effect. Generally, microalgae show growth inhibition at pH above 10–11 [44,83]. In microalgae cultivation, pH is mainly controlled by carbon species (carbon dioxide, carbonate, and bicarbonate). These species show buffering effect. Beside the effect of carbon consumption on pH, microalgae undergo different growth stages. Each stage show different pH pattern.

Medium composition is another important aspect of microalgae cultivation. Basically, there are two types of medium, natural and artificial. Natural medium is cheaper than the artificial medium, but it is not recommended because natural medium does not contain all of the required nutrients up to the desired concentration. Therefore, artificial media are introduced for cultivation. In artificial medium, the nutrients composition and their concentration are manipulated based upon the type of microalgae species. The most commonly used artificial media hu 13, Johnson's medium, Bold basal medium, F-2 medium, Beneck's medium, PES medium, Fogg's medium, Noro medium, MA medium, BG-11 medium, C medium, ASW medium, and AF6 medium. Salinity can affect biomass yield and lipid contents of microalgae. Marine microalgae for example, *Synechococcus sp*, *Nannochloropsis salina*, *Chlorococcum littorale*, *and Botryococcus braunii* can thrive in high salinity environment, whereas freshwater microalgae (*Ch. vulgaris*, *Microcystisa eruginosa*) grow in less saline medium.

4.4. Challenges and limitations of microalgae cultivation

The following challenges should be addressed for efficient cultivation of microalgae at large scale.

- Isolating microalgae species from local environment
- Selecting robust microalgae species.
- Improving light capture efficiency.
- Improving photosynthetic efficiency.
- Improving CO_2 solubility in microalgae culture.
- Improving oxygen solubility and removing oxygenic byproducts.
- Improving biomass yield.
- Improving growth rate.
- Controlling culture temperature.
- Controlling bacterial contamination.
- Maintaining carbon to nitrogen ratio.
- Removing stagnate zones (generated due to improper mixing).

4.5. Lipids production

Microalgae species vary in their biomass yield, lipids yield and composition (Table 2). Lipids quality and quantity depends mainly on the nutrients conditions. Microalgae develop lipids in the absence of nitrogen and silicon. Microalgae biomass is composed of carbohydrates, lipids and protein. Protein contents are high in exponential phase, while carbohydrate and lipids are high in stationary phase. Microalgae produce more protein in exponential phase as compared to stationary phase due to high nitrogen uptake in former case [34]. Growth rate and lipid are inversely correlated. Studies imply that 15–30% increase in lipid content would decrease the growth rate up to 50%. Rodolfi et al. viewed that 22% of the nutrients is used for biomass production in the plants and the rest is used oil production [93]. Lipids are divided into three categories: neutral lipids, crude lipids, and total lipids. Neutral lipids contain triglycerides, free fatty acids, hydrocarbons, alcohols, esters and wax. Crude lipids and total lipids contain pigments. Total lipids also possess phospholipids and glycolipids [4].

Lipids yield can be increased by regulating the nutrients. The lipids yield is mainly affected by nitrogen. In diatoms, silicon affects the lipids yield. Other nutrients such as phosphate, sulfate, and iron also play crucial role in lipid accumulation [94]. The composition of lipids changes with nutrients. Microalage show high level of C16:0 and C18:0 in nutrients deprived condition.

Table 2
Lipids and biomass productivity of different microalgae species.

Microalgae	Biomass productivity (g/L/day)	Lipid productivity (mg/L/day)
Chlorella vulgaris	0.25	54
Chlorella sp.	0.53	178
Chlorella emersonii CCAP 211/11N	0.04	49.89
Chlorococcum sp. UMACC 112	0.28	53.7
Chlorella protothecoides	4.4	3701
Nannochloropsis sp. UTEX LB 1999	0.35	109.3
Nannochloropsi oculata NCTU -3	0.48	142
Nannochloropsis sp.	0.17	60.9
Nannochloropsis sp. F&M-M28	0.17	60.9
Nannochloropsis sp. F&M-M26	0.21	61.0
Nannochloropsis sp.	N.D	76.5
Nannochloropsis sp. F&M-M24	0.18	54.8
Pavlovalutheri CS 182	0.16	50.2
Scenedesmus sp. DM	0.28	53.7
Nannochloropsis oculata	N.D	142
Neochloris oleoabundans	N.D	134
Dunaliella salina	–	116
Dunaliella teritiolecta ATCC 30929	0.10	69.8
Chaetocero scalcitrans	N.D	17.6
Botryococcus braunii	0.02	–
Pavlovasalina	0.16	49.4
Spirulina platensis	0.08	–
Isochrysis galbana	1.60	–
Chlorella pyrenoidosa	3.64	–

Yeesang et al. found relatively high level of lipid content at light intensity of 82 uE/m^2/s and 0.74 mM of iron [79]. A variation in temperature can alter the lipid composition and its total yield. Unsaturated fatty acids show high yield at low temperature and saturated fatty at high temperature [94]. Light intensity also influences the composition of lipids. High light intensity helps to develop polar and neutral lipids, whereas polar lipids are produced at low light intensity. Light intensity changes the metabolism of fatty acid formation. High light intensity (>60 μmol/me/s) alter NADPH, pH and magnesium content of microalgae biomass. It is viewed that lipid content decrease at high light intensity. This decrease occurs due to inactivation or decrease in chloroplastidial activity at high light intensity [58,74]. Yoshioka et al. (2012) found an increase in lipid content of *Isochrysis galbana* by the intermittent supplement of blue light [95]. Microalgae accumulate more lipids under high light intensity. Jiang et al. found a biomass concentration of 2.23 g/L and lipid content of 59.9% under high light and nutrients-deprived condition [96].

5. Harvesting

Recovery of microalgae biomass from the medium is called *harvesting* [97,98]. This is a key step in biodiesel production process [31]. It contributes 20–30% of total cost of biodiesel production. Small cells of microalgae (1–10 um diameter) exist in dilute culture medium having density almost equal to water. Biodiesel production from such a dilute culture is not possible. First, it needs to be concentrated up to 50–200 times.

Microalgae harvesting is a difficult process because microalgae cells possess high surface charge and remain stable in the growth medium. Destability which is the precursor of harvesting, demands state-of the-art technology. Two forces play their role in stability and settling of microalgae (1) gravity force and (2) drag force. These forces vary with the shape and size of the cells.

No universal technology has been introduced yet for efficient and low-cost harvesting of microalgae [99]. The existing technologies include, centrifugation, coagulation, flocculation, dissolve air filtration, membrane filtration, electro-flotation, electrophoresis, and ultrasound [100,101]. None of the technology has proved ideal yet. Thus there is dire need to develop cost-effective and efficient harvesting techniques. In the following section, we discuss the prospects, operation, and limitation of existing technologies of microalgae harvesting.

5.1. Centrifugation

Centrifugation can harvest microalgae by centrifugal force. A centrifugal force higher than the gravity force separates microalgae biomass from the medium. Harvesting efficiency of 80–90% can be obtained within 2–5 min operation via centrifugation [102]. There are different types of centrifuges: nozzle type centrifuge, solid-ejecting disc centrifuge, solid-bowl-decanter centrifuge, and multi-chamber centrifuge. The selection of centrifuge depends on the particle size and harvesting efficiency. These days, multi-chamber centrifuge is gaining attention due to its easy operation. In multi-chamber centrifuges, particles are separated in different chambers based on their particle sizes. Nevertheless, their large scale application demands high investment cost [103].

5.2. Filtration

Filtration is a widely used technology in microalgae harvesting. In filtration, microalgae suspension is passed through a porous medium. The particles less than the pore size are filtered out, and the residual particles retain on the filter. The pore size of the porous medium or filter depends on microalgae species [102]. Microfiltration (0.1–3 μm pore diameter) and ultra-filtration (0.1–0.01 μm diameter) techniques are the best suited for microalgae harvesting [104]. The harvesting efficiency of 95% can be attained through these filtration techniques. The harvesting efficiency is determined by flux passing through the filters. High flux does not necessarily means high harvesting efficiency. At low concentration of microalgae culture, flux would be high but harvesting efficiency can be low. The presence of extracellular polymer substances (EPS) in microalgae growth medium can reduce harvesting efficiency [61,105]. EPS deposit on the filter surface and cause bio-fouling. Some other factors such as microalgae surface charge, contact angle, cell size, culture age, membrane type (hydrophilic or hydrophobic), temperature, and microalgae culture concentration also influence filtration process. Above all, fouling is the major bottleneck of filtration process. Due to fouling, replacement of membrane and backwashing are required which increase the overall process cost. Addressing the issue of fouling can prove filtration as an ultimate harvesting technology. Fouling can be reduced by applying pressure across the filter. Uduman et al. have demonstrated that pressure application is only suitable for large sized species, but in-efficient for micro-sized algal cells [102]. Altering the filtration mode, dead end filtrations to cross-membrane filtration, can also reduce fouling. In cross-flow

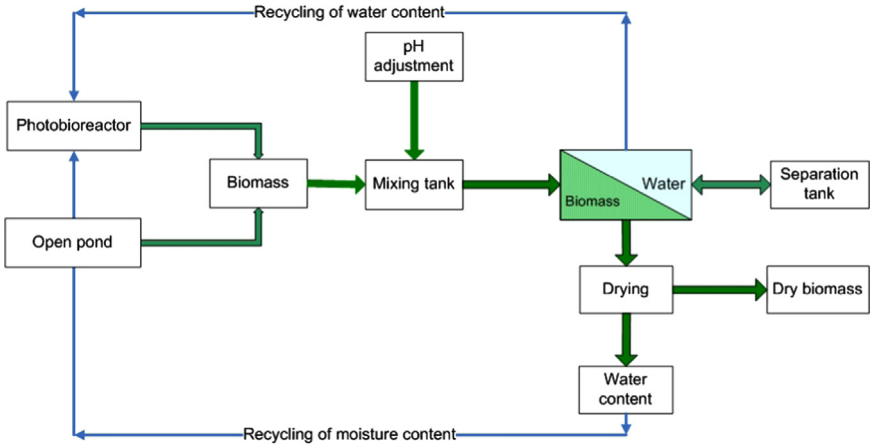

Fig. 3. A process of microalgae harvesting by flocculation.

membrane filtration, relatively high fluid velocity, pressure, and backwashing help to reduce fouling. Membrane surface modification can warrant developing an efficient microalgae filtration system in future.

5.3. Flotation

In flotation, microalgae are separated from aqueous medium by introducing air bubbles. Air bubbles trap microalgae cells and move upward due to buoyancy and low density effect. Microalgae cells stay at top surface and can be collected by skimming. The cells having a diameter of 10–30 μm are efficiently removed by flotation. Studies show that 80–90% harvesting efficiency can be achieved by this method. Flotation depends on the size of microalgae cells and air bubbles [106]. Small cell size can easily be carried out by air bubbles showing high flotation efficiency. They possess high area-to-volume ratio, efficiently attach with microalgae cells and have high stabiliy [65]. Air flow rate, hydraulic retention time, and surface characteristics of microalgae also affect flotation process. Flotation can be carried out by various methods: (1) dissolved air flotation, (2) dispersed air flotation, (3) ozone flotation and (4) electro flotation.

In dissolved air flotation, liquid stream saturated with dissolved air is injected in microalgae suspension through nozzle. Air bubbles generated from the nozzle attach with microalgae cells and rise to the surface. In contrast, dispersed air flotation involves the injection of non-pressurized air into microalgae suspensions. Dispersed air-flotation generates larger bubbles than dissolved air flotation, exhibiting lower flotation efficiency. In ozone flotation scheme, ozone disrupts microalgae cell wall and release protein. Released protein acts as a bio-flocculant. Recently, electro-flotation is getting widespread attention [102]. In this technique, hydrogen gas bubbles are generated by the electrolysis of water. Hydrogen bubbles effectively carry microalgae cells to the surface for skimming. Flotation is advantageous over other harvesting methods. It is cheap, simple in operation and less time consuming but scaling of electrodes and high power input limits its application at commercial scale.

5.4. Flocculation

Flocculation is a process in which the particles aggregate with each other to form flocs. High density forces the flocs to move downward and settle at the bottom as sediments [107,108]. In microalgae growth medium, the cells are negatively charged and present in stable form. Microalgae cells must be destabilized first to separate them from the aqueous medium [109]. The cells can be destabilized by their interaction with counter positive charge. Fig. 3 illustrate the process of microalgae harvesting by flocculation. A number of flocculants have been identified for this purpose. Metal ions, (Al^{+3} and Fe^{+3}) are commonly used flocculants in water treatment and also for microalgae harvesting. Metal ions work efficiently; however, their high concentration can contaminate the harvested biomass [110,111]. Also, their high concentration disrupts the cell wall [112]. Cell lysis and interference in lipid extraction are the major concerns in using such flocculants. Alternatively, organic polymers such as chitosan, cationic starch, grafted starch, and cationic starch can be used [113]. These flocculants show high efficiency, and do not contaminate the biomass [31]. The effect of organic flocculants on lipid extraction is not widely studied. Organic flocculants show high efficiency but they work at low pH, and their application is not economical. The use of oxidants (O_3, ClO_2) aids in flocculation process [62,81,114]. A small amount (~3 mg/L) of potassium permanganate can improve the flocculation process, however, it can break the cell wall which results the release of intra-cellular compounds [115]. In the presence of intra-cellular compounds, the flocculant demand increases. Oxidants have ability to destabilize the suspended particles and EPS in microalgae culture [116]. Petrusevski et al. demonstrated that the oxidant change the cell surface which improve their ability to bind with the flocculant [87]. The rate of sedimentation is also controlled by seasonal changes, mass balance of the nutrients, and contamination by other microorganisms [117].

Most recently, the concept of auto-flocculation is becoming popular. In auto-flocculation, microalgae are harvested without any flocculant aid [62,118]. Auto-flocculation occurs by change in pH during microalgae growth [51,112]. Microalgae cells have natural tendency to flocculate at pH 9 or above. Low CO_2 supply, long cultivation time also causes the cells to settle.

Salim et al. proposed to optimize the ratio of flocculating and non-flocculating microalgae to decrease the cost and energy consumed in harvesting process [118]. Co-culturing of filamentous fungi with microalgae is an effective approach for effective harvesting. Fungi have ability to degrade cellulose, present in microalgae cell, and use them as food. As a result, microalgae pelletize to harvest. Similarly, oleaginous microbes can be co-cultured with microalgae. These microbes produce sugars which are converted into lipids by microalgae. On the other hand, oleaginous microbes aid in making flocs with microalgae. Jianguo et al. co-cultured *C. vulgaris* with different microorganisms [119]. They found 100% harvesting by growing *C. vulgaris* with *Mortierella isabellina*. Salim et al. studied the harvesting phenomenon by mixing auto-flocculating microalgae with non-flocculating microalgae species [118]. They found 25% increase in biomass recovery by mixing *C. vulgaris* (none-flocculating microalgae) with *Ettliatexensis*, *Ankistrodesmus falcatus*, *Scenedesmus obliquus* (flocculating microalgae) with a ratio of 0.25. Microalgae release EPS in thes medium. EPS act as bio-flocculants. They may obstruct the flocculation process, depending on their concentration and microalga species [120] *Dictyosphaerium pulchellum* showed high flocculation efficiency at 0.1–0.2 mg/L of EPS as carbon. However, the efficiency decreases at concentration > 1–2 mg/L [121]. Flocculation is affected by algogenic organic matter (AOM). Microalgae release AOM by metabolic excretion or by the breakage of the cells. AOM are composed of lipid, polysaccharides and nucleic acids. AOM with high molecular weight have positive impact on flocculation while low-weight AOM decrease the flocculation efficiency. Studies demonstrate that protein content can interfere in flocculation process [100,121]. It is demonstrated that the presence of bacteria or fungi in microalgae growth medium helps to induce flocculation. Actually, fungi have net positive surface charge which interacts with negatively charged microalga cells to form flocs [122]. Light is the key element of auto-flocculation. EPS are produced in the presence of light which cause cell aggregation. The cells produce high EPS under high temperature also. Zhang et al. noticed an increase in EPS with microalgae culture age [65].

Bio-flocculation is somewhat related to nutrients consumption. In nutrients (nitrogen and phosphorous in particular) deficient condition the cells produce EPS. Generally, the cells consume 6.5–100 times more CO_2 than nitrogen and phosphorous. Hu et al. found a direct correlation between CO_2 consumption and EPS production. Mixing of different microalgae can also induce flocculation [123]. Studies have demonstrated that auto-flocculation of microalgae varies with its growth stages, the highest in stationary phase and the lowest in exponential phase. The flocculation behavior of microalgae highly depends on its algal group. Microalgae vary in their cells wall structure and size. Eldridge et al. have concluded that microalgal species of same size belonging to same taxonomic class even show variation in flocculation behavior [124]. Likewise, microalgae species belonging to different groups have varied cell size and thus variation in flocculation efficiency.

Therefore, it is recommended to study the flocculation behavior of different microalgae separately instead of assuming their general trend. Microalgae cells occupy different surface charge depending on the dissociation of acids and AOM composition. It is reported that *Syneschocystis* and *Chlorella* release AOM up to 1.8 mg/L and 81 mg/L respectively. AOM concentration increase over the time of cultivation [101].

Recycling of harvested biomass is another approach to improve the economics of microalgae harvesting. Genetic modification is a tool to enhance auto-flocculation potential of microalgae [42]. By genes modification, it may be possible to develop such species which express protein in cell wall, or wall-deficient microalgae [114,116]. Understanding the dynamics of microalgae cultivation can improve its auto-flocculation ability. Manipulating the effect of light and nutrients on flocculation can lead to find high flocculating microalgae. Flocculating microalgae would serve as flocculant for non-flocculating cells. Gonzalez-Fernandez et al. hypothesized to introduce filamentous microalgae in the culture medium during late-exponential phase [125]. The micro-organisms such as fungi and yeast are being explored which assist in microalgae harvesting. This method is known as bio-flocculation. Bio-flocculation of microalgae is a complex phenomenon. As discussed earlier, the major driving force to execute bio-flocculation is EPS. EPS have high binding ability [92]. The cells in microalgae culture are surrounded by EPS. In the presence of EPS, more ions are generated and zeta-potential increases. Low zeta-potential is required for flocculation process. Divalent ions also play a vital role in bio-flocculation. Positive charge on divalent decrease the zeta-potential. In heterotrophic cultivation, microalgae take up organic compounds; activate the pumping of proton into the cell. The composition of floc and storage of polymer within the cell varies with the organic substrates [40]. The dose of organic substrate and their loading pattern govern significant changes in floc properties. Finding a correlation between coagulant demand and surface area of the cells can give an insight of flocculation phenomenon.

For efficient bio-flocculation, the effect of pH, temperature, mixing speed, carbon to nitrogen ratio should be explored [80,105]. At low pH, the cells get protonated and repel each other; thereby flocculation can not take place completely. At high pH, the cells are occupied by anions. Anions also repel each other and thus flocculation does not occur. At optimum pH, the cells and the flocculant are optimally charged to form stable flocs. Rashid et al. study supported such mechanism. The highest efficiency (99%) was achieved at pH 6 [126].

Temperature also affects bio-flocculation process. Microalgae cells grown at different temperatures show a variation in flocculation efficiency. The temperature of microalgae culture also affects harvesting efficiency. Dissolve oxygen (DO) is closely linked with temperature, which in return influence growth. Barbosa et al. showed high sludge settling and DO 0.5–2.0 mg/L. High DO break the flocs [127]. The cells size, structure and morphology changes with temperature. At low temperature, the flocculant can not mix properly with the cells. At low temperature, hydrolysis decreases and the time of coagulation prolongs. However, flocculation completes in shorter time. The effect of temperature on flocculation process can vary with the flocculant type [128]. Yet very little information with regard to the effect of temperature on flocculation is available in the literature.

5.5. Harvesting by polymers

Coagulation/flocculation is a polymer-induced phenomenon [124]. The polymers results high harvesting efficiency and low sludge volume. Polymers used for microalgae harvesting have high charge density and are water soluble. Polymers are generally divided into three categories (1) non-ionic, (2) cationic, and (3) anionic. Non-ionic polymers are used in water treatment process, but their use in microalgae harvesting is rare. Pomyacrylamide, starch, cellulose, gelatins are well-known non-ionic polymers. As the name indicates, anionic polymers contain net negative charge on their surface (Table 3). These are prepared by polymerization of acrylamide and acrylic acid. The charge density of anionic polymers ranges from 1.4 meq/g to 10.6 meq/g. As name indicates, cationic polymers contain positive charge. The most commonly used cationic polymers include dimethyamine polymers, cationic polyacrylamides, chitin and chitosan [129]. These polymers vary in their charge densities ranging from 1.2 meq/g to 7.3 meq/g. The structure of polymers and their efficacy are pH dependent.

Polymers can be further divided as synthetic and natural polymer. Synthetic polymers show high efficiency, produce big and stable flocs, and require less dose. Despite these characteristic of synthetic polymers, they are toxic and give poor quality of microalgae after harvesting. The level of toxicity varies with the type of the polymer. As cationic polymers show the highest level of toxicity while non-ionic polymers are the least toxic. Natural polymers are non-toxic and biodegradable [32]. Natural polymers are also termed as *bio-flocculants*. Now a days, chitosan is grasping widespread attention as a bio-flocculant [130]. Chitosan is a polymer of D-glucosamine, produced by the deacetylation of chitin [131]. Fungi are also reported to produce chitosan under anaerobic condition [126,131,132]. Chitin is the second most abundant polysaccharide in the universe [129]. Chitin is composed of N-acetylglucosamine. It is found in invertebrates and crab shells [133]. To obtain chitin from crab shell, it undergoes the step of pretreatment, deacetylation, demineralization, dewatering, and color removal [134,135]. Once chitin is achieved, it is deacetyled, washed and de-watered to get chitosan. Chitosan contain reactive amino, and hydroxyl group [133]. Chitosan has been used, as a flocculant, for the treatment of wastewaters including food waste, textile waste, pulp and paper waste, and brewery wastewater [134]. Chitosan is non-toxic, biodegradable, environmentally friendly, and has strong binding ability [136]. The performance of chitosan depends on its degree of deacetylation, molecular weight, and solubility [133,135]. Chitosan is insoluble in water, and soluble in acids. Insolubility occurs due to the presence of hydrogen bond. The solubility depends on degrees of acetylation (DA). Chitin and chitosan have DA of 0.9 and 0.35, respectively. DA varies with amino-group. DA proportionally varies with amino group. In addition to amino group, hydroxyl radical in chitin and chitosan change their solubility. Chitosan can be dissolved in acetic

Table 3
Polymers used for microalgae harvesting.

Polymers type	Polymer name
Cationic polymers	Poly-ammonium chloride, poly-aluminum chloride, cationic polyacrylamides, di-methlyamine polymers, di-methylamine polymers, polystyrene, chitosan, ionenes,
Anionic polymers	Lignin sulfate, anionic polyacrylamides, carboxylic acid
Non-ionic polymers	Polyacrylamide, starch, cellulose, chondroitin sulfate,dextran sulfate, heparin, tannis

acid, formic acid, nitric acid, citric acid, and hydrochloric acid [113]. The solubility of chitosan varies with acids. It shows different crystalline structure and water sorption capacity. The basic criterion to enhance the solubility is to convert β-chitin into α-chitin. The solubility depends on pH. At low pH, amine group of chitosan get protonated which is water soluble. It is a general idea to add proton concentration equal to amine concentration. At high pH, amine group can deprotonate making the chitosan insoluble. Amine gets protonated at low pH. Chitosan dissolves at pH 6. To improve the solubility of chitosan, it is chemically modified [130]. Chitosan grafted with N-vinyl pyrrolidone show relatively high solubility than pure chitosan. The solubility varies with its degree of de-acetylation, charge density, and molecular weight of chitosan. It is established that the performance of chitosan is proportional to charge density and degree of de-acetylation. Chitosan derived from various sources also show variation in solubility and thus flocculation efficiency. Rashid et al. found that chitosan solubility is an indirect measure of flocculation efficiency of microalgae [126]. High solubility induces high flocculation. They measured the harvesting efficiency of microalgae by dissolving chitosan in hydrochloric acid, nitric acid, citric acid, and phosphoric acid. The order of performance was hydrochloric acid > phosphoric acid > nitric acid > citric acid. Hu et al. tested the chitosan solubility by applying different concentration of acids. The solubility was the maximum at 0.05–0.15 mol/L of acids [94]. Four mechanisms are identified for microalgae flocculation (1) charge neutralization, (2) patching, (3) bridging, and (4) sweep flocculation [122].

In charge neutralization, surface charge of the particles is reduced or neutralized by adding polymer. The polymer interacts with the particles by reducing electrostatic repulsion. The dose of polymer depends on charge density. Polymers with high charge density induce flocculation at low dose because they can provide more charge. Polymer is added until zeta-potential reaches to zero. The attraction forces outweigh repulsion forces to induce flocculation. High concentrations of positively charged generate a net positive charge on the cells. These cells attract others cells to form flocs. This phenomenon is called patching. The flocs formed by this phenomenon are weak. Microalgae are harvested by sweep flocculation phenomenon when flocculant concentration is relatively high. Microalgae cell are trapped by the flocculants to form precipitates. Bridging is the mechanism in which a long chain flocculant bind with the particles in multiple directions, and form bridge like structure. This is a reversible process. At optimized polymer dose, all empty sites of the particles are covered by the polymer to make stable flocs. However, at high polymer dose, the particles surfaces are in-sufficient to interact with the polymer. As a result, electrostatic interaction decreases and flocculation halts.

5.5.1. Electrolysis

In this process, ions are generated by the electrolysis of water or metals. Electrolysis is divided into two categories: (1) electrolytic coagulation and (2) electrolytic flocculation. In electrolytic coagulation, positively charged coagulants are used at sacrificial anode [128]. The coagulants form a complex with hydroxide ions to react with microalgae cells. In electro flocculation no coagulant is involved and aggregates are formed by directing the microalgae cells towards anode. Electrolysis can harvest microalgae up to 95%. However, the harvesting of marine microalgae through electrolysis shows low efficiency. High ionic strength of seawater decrease the harvesting efficiency and high energy input [20]. Microalgae in saline water require ten-fold more energy to flocculate than freshwater. Low coagulant dose, rapid harvesting, and its working at natural pH are added advantages of electrolysis. However, high cost and scaling of cathodes and low efficiency for marine microalgae are major bottlenecks for its large-scale application.

5.6. Ultrasound

Microalgae can be harvested by ultra-sonication at low frequency and low amplitude. Sonication disrupts microalgae cells, decrease their buoyancy, and increase the ability to settle down. Studies show the harvesting efficiency of 90–92% by sonication [109]. Sonication applied even for short time (5 s only) can show high harvesting efficiency. Sonication at high frequency and amplitude can be deleterious as it rupture microalgae cells and release lipids into aqueous medium [137]. Ultra-sonication is difficult to commercialize because of high energy input.

5.7. Limitations and challenges of microalgae harvesting

The major limitations and challenges encounter in microalgae harvesting are the following:

- Stable and dilute microalgae culture.
- Small cell size.
- Versatile nature of microalgae cell size, shape, and motility.
- Presence of AOMs in microalgae suspension.
- Contamination of harvested biomass by chemicals.
- Interference with lipid extraction.
- Different protocol for freshwater and marine algae.
- No single methods applicable for all types of microalgae culture.
- Low harvesting efficiency.
- High energy input.
- High flocculant dose.

Table 4 presents a road map summarizing the potential barriers, solutions, future interests, and R&D goals of microalgae harvesting.

6. Oil extraction

The harvested biomass of microalgae undergoes further processes for oil extraction. Oil extraction is a critical step in biodiesel production process [138]. Oil in microalgae is encapsulated by rigid cell wall composed of carbohydrate and protein. Therefore, the disruption of cell wall is one necessary step to extract oil from microalgae. Oil extraction is an expensive and challenging step. No single method of oil extraction is applicable to all species of microalgae because of variation in their microalgae cell structure and size. Various methods, mechanical, chemical, and biological are used for cell lysis. In the following section, the methods of oil extraction, their advantages and limitations are discussed.

6.1. Mechanical

Mechanical methods are widely used for oil extraction whereby the cells are disrupted by physical force [109]. The efficiency of oil extraction through this process depends on microalgae species. For example, *Spirulina sp.* do not have rigid cell wall and easier to oil extraction than *Chlorella sp*. Mechanical disruption can be carried out by various methods such as ultra-sonication, bead beating, and microwave. Each technique has some pros and cons.

Bead beating is a simple and rapid method of cell disruption. In this method, the cells are disrupted by their collision with metal beads. Studies have demonstrated that the efficiency of these methods depends on reactor size, bead characteristics, and shaking speed. Based on recent available studies, the efficiency of this

Table 4
Roadmap for microalgae harvesting.

Process	Technical barriers in harvesting techniques	Action required	Recent Trends	R & D goals
Harvesting	**Flocculation** • Low harvesting efficiency due to small algal cell size • Harvesting techniques depend on strain selection • Less efficient for marine algae (due to high salinity) • Flocculants recovery for cost reduction and water purification • Increased environmental pollution due to excessive use of flocculants or additives	Optimize flocculation method, culture pH manipulation and its impacts on environment should be understood	– Currently, the alternate of the conventional harvesting techniques such as flocculation, coagulation and sedimentation are being explored owing to several drawbacks such as high cost involved in centrifugation, recovery of salts and water purification in flocculation technique, and inefficient designs of sediments tanks. – The interest is growing in dissolved air flotation, electrophoresis, auto-flocculation and bio-flocculation – The manipulation of sonication and metabolic engineering hold great promise to prove effective for microalgal harvesting	– Reduce process energy and cost – Investigate the feasibility of recent harvesting techniques – Assess each technology option in terms of overall system compatibility and sustainability – Introduce novel harvesting techniques
	Filtration • Non-availability of proper filter material • Optimized design required to avoid cake formation in filtration process • Dependent on microalgae strain • Expensive	Optimize filtration design; filter material and the concept of vibrating filters need to be introduced.		
	Centrifugation • High capital cost	Low cost commercial units should be introduced		
	Sedimentation • Lack of proper design	Optimized sediments tanks with high flocculants recovery and water recycling are to be designed		
	Dissolved air flotation • No significant studies available	To establish baseline studies as well as engineered design to figure out optimal bubble size and their distribution in culture		
	Electro flotation/coagulation/ flocculation • Periodic replacement of electrodes • Scaling of electrodes • Only few studies available	To devise an effective technique to remove scaling from the electrodes		
	Auto-flocculation • Limited ability of microalgae to uptake CO_2, P and other nutrients • Correlation of pH, nutrients uptake and flocculation is not developed so far	To investigate the interaction of nutrients uptake, pH change and oxygen dissolution		
	Bio-flocculation • Development of synergistic relationship between bacteria and algae	To develop a synergetic relationship b/w bacteria and algae and its comparative techno-economics analysis with other harvesting techniques		

- Techno-economics analysis is not available

Sonication Intensive studies required to exploit its effectiveness
- Not much studied

Metabolic manipulation Intensive studies required to improve the energy contents of microalgae
- Development of high energy strains

method is quite low. Also, high energy input and heat dissipation during the operation reduce its application.

High intensity of ultra-sonication (400–800 KHz) produces micro-bubbles, heat, and shockwaves which are helpful to break microalgae cells wall. Ultra-sonication is considered as the best method for cells disruption, but it can not be applied on all species of microalgae. The efficiency of ultra-sonication depends on various parameters such as micro bubble size, medium density, microalgae cell concentration, temperature, and frequency. Ultra-sonication is long-known technology and efficient too. However, high energy consumption and heat dissipation are major bottlenecks for its large-scale application.

6.2. Chemical

Cell disruption can be induced by chemical methods also. Commonly used chemicals are chloroform, hexane, benzene, acids and alkalis. Chemical extraction can be carried out in wet or dry microalgae biomass. Dry biomass shows high extraction efficiency. Chemicals show high permeability in dry biomass than wet biomass. Water molecules in wet biomass weaken the interaction between the chemicals and microalgae cells. Considering the high cost of drying, extraction from dry biomass is not economical. Regardless of high efficiency through chemical extraction there are some limitations. Low cell concentration of microalgae biomass requires high concentration of chemicals for disruption, increasing the overall cost of extraction process. Also, chemicals can change the composition of fatty acids. Rawat et al. have suggested that modification in equipment design, operating condition, and applying the optimal combination of polar and non-polar chemical can improve the efficiency of chemical extraction [20].

6.3. Other methods

Oil extraction can be carried out using osmotic shocks and by using enzymes. In osmotic shock, the cells are disrupted by developing a pressure across the cell wall [109]. High salt concentrations in liquid medium develop a pressure on the cell wall, called *hyper-osmotic stress*. A continued pressure on cells wall forces it to bounce outward (towards medium). Likewise, pressure can develop if salt concentration inside the cells is higher than the liquid medium. The use of enzymes for cell disruption is a promising approach. The enzymes break the sporo-pollenin layer of the cells only without harming the structure of the whole cell. In contrast to chemical methods, the enzymes do not interfere with fatty acids. Most abundantly used enzymes are cellulase, neutrase, pectinase. Enzymes show high extraction efficiency but they are not cheap. Recently, the idea is coined to extract oil directly from the live cells. This technique is termed as *milking*. In milking process, an organic solvent is introduced in growth medium of microalgae to extracts lipids from

Table 5
A comparison of microalgae cultivation in photo bioreactor (PBR) and open pond system (OPS).

Parameter	PBR	OPS
Contamination	Less	High
Mixing	High	Less
Capital cost	High	Low
Area	Low	High
Evaporation	Less	High
Gas exchange	High	Low
Lipids yield	High	Low
Biomass yield	High	Low
Commercialization	Difficult	Easy
Harvesting	High	Low
Process optimization	Easy	Difficult

the cells. Organic solvent must not be toxic for the live cells. So far, very little information is available in literature about this method; therefore the effectiveness of this method is questionable.

6.4. Limitation and challenges of oil extraction process

The major challenges in oil extraction process are as follows:

- Rigid cell wall.
- Weak interaction of chemicals in wet biomass.
- Low extraction efficiency.
- High drying cost.
- High consumption of chemicals.
- Reaction of chemicals with other products.
- Separation of lipids from liquid medium.
- Dependence on physical properties of the cells.

7. Future prospects of microalgae biofuel

As interest is growing to develop cost-effective process of microalgae biofuels [22,139]. A rigorous research is needed in some areas to establish a consolidated scheme for efficient production of biofuels. In microalgae-based biofuels production process, specie isolation and selection should be researched in detail. Local species possessing high growth rate and lipids yield, can be identified [23]. Benemann has iterated to isolate "domesticated" strains of microalgae which have potential to grow in open pond. Microalgae cultivation can be carried out open ponds or photo-bioreactors. Both systems have their own pros and cons. s Table 5 illustrates the limitations of microalgae growth in photo-bioreactors and open pond system. In this perspective, *Micractinium*, *Nannochloropsis*, and *Botryococcus braunii* have been identified as potential species [140]. *Micractinium* can grow efficiently in wastewaters. *Nanochloropsis* and *Botryococcus braunii* are reported to be environmental resistant and can grow in open pond system. They give high oil yield (60–80% of cell biomass). They are able to grow in critical weather conditions with fluctuating light, temperate, pH and oxygen level. Resistant microalgae species against viruses, fungi and grazers are required to grow microalgae in wastewater. Considerable attention should be paid to increase photosynthetic efficiency of "domesticated" species by applying genetic engineering approach. A mathematical model can be applied to evaluate the performance of selected microalgae specie based on strain stability, growth rate, lipid yield, photosynthetic efficiency and tolerance to the environment. Auto-trophically grown species show high photosynthetic efficiency and growth rate. However, the supplement of light and nutrients make it economically un-competitive. The issue of light supplement can be resolved to some extent by using sunlight or by designing light-efficient photo-bioreactor. The nutrient can be reduced by using wastewater as a growth medium. Pre-treatment of wastewater is necessary before feeding it to microalgae, which is quite expensive step [24]. Another issue in using wastewater as a growth medium is contamination. To date, no superb technology has been developed to control contamination. Research should be carried out to characterize different types of wastewaters suitable for microalgae cultivation. Moreover, low-cost methods should be devised to control contamination.

Harvesting also is a challenging step. Self-flocculation or bio-flocculation is the most recent method being proposed for efficient and low-cost harvesting [119]. This technology is quite mature in the perspective of wastewater; however, its applications in micro-algae perspective are not exploited yet. Finding self-flocculation ability of different microalgae species can be a promising area of research to work. Promoting mixed culture (growing different species together) cultivation may bring significant improvements in self-flocculation process. The self-flocculation ability can be increased by manipulating culture conditions. Further research should be carried out to unravel this phenomenon.

Lipids extraction is a critical step in biodiesel production process [20]. The existing methods of lipid extraction result low biodiesel yield. The use of chemicals for lipids extraction contaminates the cell biomass making it unfit for further use. Also, there are some technical limitations. Advancements in existing methods are inevitable to improve process efficiency. Lipid extraction through enzymes can be an appropriate choice. Presently, the concept of simultaneous harvesting and extraction is being widely studied. Fig. 4 summarizes the parameters affecting biodiesel production process.

Based on above mentioned discussion, it can be concluded that economical production of microalgae biofuels can not be deemed without integrating it with other technologies. Recently, micro-algae are being promoted to use in microbial fuel cell (MFC) as an anodic feedstock. Firstly, oil is extracted from microalgae biomass and spent biomass containing sufficient amount of carbohydrates and proteins, is used at anode [141–143]. Rashid et al. have developed a closed circuit MFC. In this MFC system, microorganisms at anode use spent microalgae biomass producing CO_2. CO_2 is transferred into cathode chamber. Fresh microalgae are grown at cathode to fix anodic CO_2. Microalgae use CO_2 to promote its growth [144]. Extensive studies have been carried out to use microalgae for wastewater treatment and energy production simultaneously. High concentration of amino acid in wastewaters is useful for microalgae. Microalgae can successfully reduce chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Microalgae are also reported to treat electroplating wastewater [7,145–147]. They can remove heavy metals from 50% to 99% (depending on metal concentration). However, pretreatment is a major barrier in using microalgae for this purpose. Pretreatment is a costly step, and impact downstream processing of microalgae biofuels.

8. Economics of microalgae biofuels

Since last decade an interest has developed for biofuels production using microalgae. A big share of research funding has been invested to promote microalgae in industries, academics, private and government owned institutions and other R&D sectors. Commercial application of microalgae biofuels demand another decade. The cost of microalgae biofuels must be significantly reduced to promote their application. According to an estimate, the cost per barrel of algal oil is US $ 300–2600. Another study reveals that the cost of microalgae oil will be double than petroleum. Chishti et al. estimated the cost of microalgae oil as $ 2.80 per liter, without including the distribution, marketing and taxes. Microalgal oil is not affordable at current level of price. Thus there is urgent need to improve the economics of microalgal biofuels. The cost of microalgae biofuels can be improved by optimizing the whole process of biofuels production. The cost of microalgae biofuels mainly depends on its cultivation. In cultivation, carbon supply is the most important nutrients source which controls the growth rate and lipid productivity of microalgae. Organic and inorganic carbon sources incur different cultivation cost. Suali et al. have proposed to use sweet sorghum [139]. The cultivation cost of sweet sorghum ranges from $0.027 to $0.48 [29,48]. Microalgae using 25–50 g/L of sweet sorghum has been reported to produce lipids contents up to 73% of its biomass. Crude glycerol is another cheap carbon source. It can be obtained from waste vegetable oil. 70–100 g/L of glycerol in microalgae cultivation is found to enhance lipids up to 73%. Flue gases are reported to be the cheapest carbon sources [139]. Microalgae can

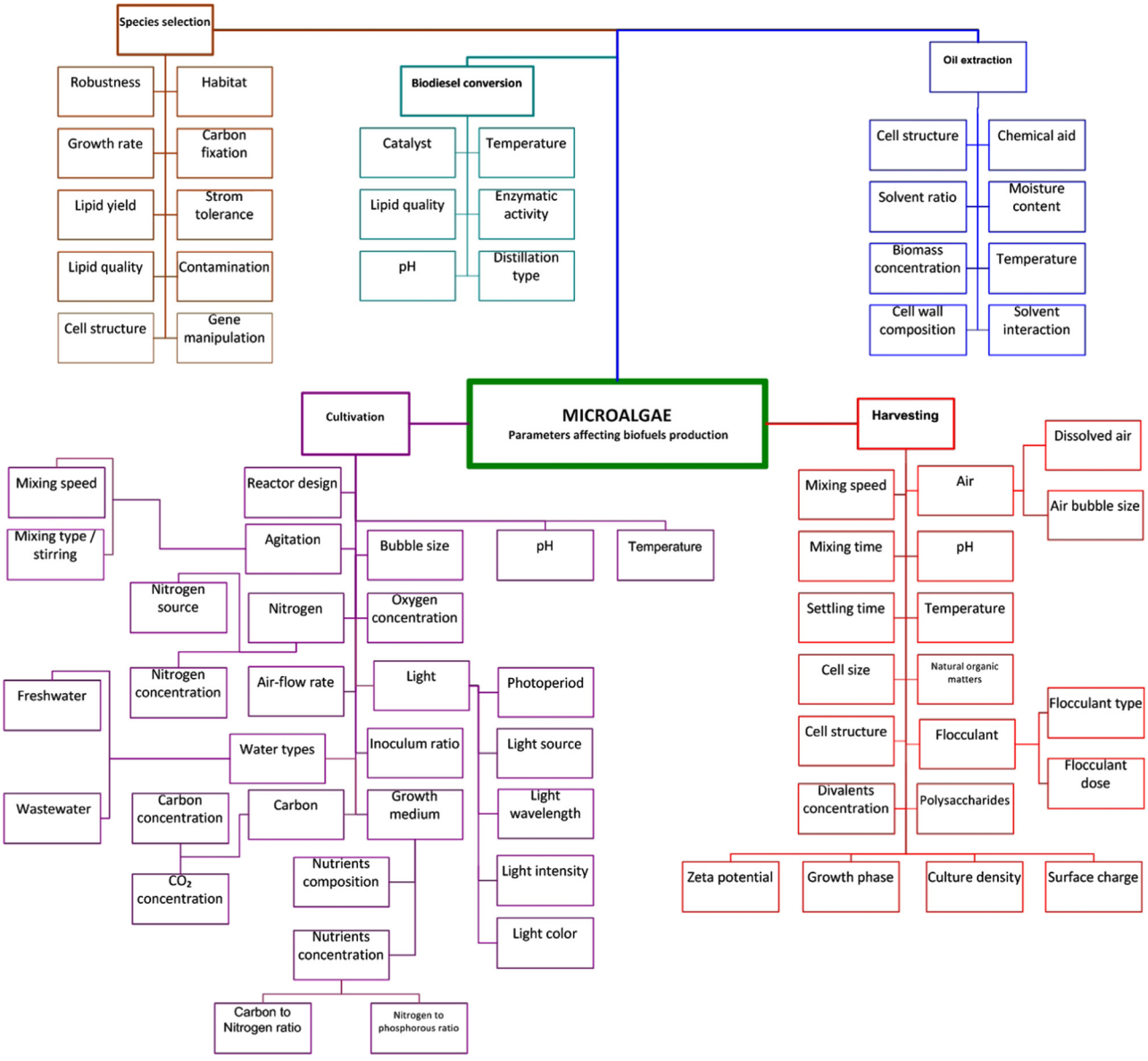

Fig. 4. Parameters affecting microalgae-derived biodiesel production process.

fix CO_2 up to 10%. Further exploring this potential of microalgae CO_2 fixation can curtail nutrients cost and reduces green house emissions [20].

Microalgae cultivation can be carried out in photo-bioreactors (PBR) or open pond system. PBR demand high capital and operating cost as compared to OPS. Benemann et al. estimated the cost (including operating and labor) of PBR two-times than open pond system. Kirrolia et al. compared the cost of photo-bioreactors, raceway ponds and fomenters [148]. The cost per kg of algae oil was $24.60, $7.64, $1.54 for photo-bioreactors, raceway pond, and fomenters respectively. The cost of microalgae biomass per kg was $7.32, $1.54, and $1.02 for photo-bioreactors, raceway pond, and fomenters respectively. Currently, fermenters are the most economical for microalgae biomass production.

The cultivation cost can be reduced by growing microalgae in brackish or saline water. Microalgae grown in brackish water are reported to exhibit high performance in open ponds as well as photo-bioreactors. Further improvements in microalgae cultivation are possible by selecting appropriate species; species possessing high growth rate, lipid productivity and resistant to the environment. Benemann et al. have viewed that wild type microalgae species can not show the desired results and are not suitable to grow in open ponds [140]. Desired properties of microalgae can be introduced by adopting several techniques such as mutagenesis and genetic engineering. Advancements in entire process, including harvesting, lipid extraction, and biodiesel conversion are required to prove the economic viability of microalgae biofuels.

9. Conclusions

Biodiesel production from microalgae has received wide spread attention. Efforts have been made for economical biodiesel production in last few years. However, there are some technical barriers which limit the production of biodiesel at large scale. Significant improvements are required in the entire chain of biodiesel production process to overcome such barriers. The major steps in biodiesel production process are: species isolation, cultivation, harvesting and extraction. There is need to find robust and tolerant microalgal species which can grow in local environment. Slow growth rate and

low biomass yield are the major challenges in cultivation process. Growth rate and biomass yield can be improved by manipulating the effect of light and nutrients. So far, much work has been carried out on autotrophic cultivation which gives relatively low biomass yield. Also, it's not economical due to high cost of light and nutrients. Thus future research should be focused on heterotrophic cultivation. Optimizing key factors of microalgae cultivation can also help to bring down the process cost.

Low harvesting efficiency is another challenge for economoical biodiesel production. The existing harvesting techniques are expensive or they cause biomass contamination. In future, the concept of bio-flocculation can receive more attention. Exploration of efficient bio-flocculants can considerably lower the cost of harvesting process. The idea to integrate microalgae cultivation with harvesting by the aid of bacteria should be researched further. Low oil extraction efficiency also increases the cost of biodiesel. Extracting oil from wet microalgae biomass, called milking, can be an attracting choice; however, this idea is rarely studied. Keeping in view, the current developments and issues in microalgae technology; low-cost biodiesel production cannot be deemed, unless it is integrated with other technologies. The use of microalgae for bio-refinery, waste water treatment, animal feeding, and fertilizers should be promoted. Most recently, interest is growing to use microalgae for CO_2 fixation and electricity generation in microbial fuel cells. In future, investment in microalgae technology, research and developments may propose new approaches to lower the cost of microalgae biodiesel.

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